Nakeirah Christie
The University of the South
Summer 2013

This summer I continued my research in the chemistry lab at Sewanee. In Advent 2012, I began working with Dr. Kikis to study the ataxin-3 protein. The aforementioned protein normally functions within the ubiquitin signaling cascade, a molecular pathway within the cells that marks other proteins for alterations or degradation. Within the DNA that codes for the protein (the ATXN3 gene) is a number of trinucleotide repeats: 'CAG.' Now, while a certain number of repeats, the corresponding protein functions properly as discussed previously. However, once the number of trinucleotides repeats goes above approximately forty-five, the protein has a toxic gain of function that is linked to Machado-Joseph Disease (MJD). Symptoms begin with ticks and twitches, but as the disease progresses, the victim falls into paralysis, eventually succumbing to the disease with difficulties with vital functions such as swallowing. There seems to be a direct correlation between number of excess repeats and severity of the onset of symptoms, but beyond that, how the protein functions in the disease has yet to be understood.

Dr. Kikis, my fellow labmates, and I worked on further characterizing the ataxin-3 protein in hopes to better understand its role in MJD. We studied its effects in the body wall muscle cells of *C. elegans,* a transparent nematode that grows to be a millimeter long. Due to the organism's short life cycle, transparency, and a plethora of well-established work on the organism , *C*. *elegans* makes an ideal model organism.

Previously, I had imaged worm strains that expressed *atx-3* protein via an extrachromosomal plasmid adjoined with YFP, protein found in jellyfish that is bioluminescent. To better understand the protein, the Kikis labs works with *atx-3* proteins with various amounts of poly-Q expansions: Q45 and Q63, with the number referring to respective number or repeats. For our controls, we likewise examined a worm that expressed YFP alone with no corresponding *atx-3* protein, and a wild-type (N2) *C. elegans*.

This summer, I was tasked with two objectives: to measure the amount of *atx-3* protein in each of the four strains, and to study the effects of motor-coordination the protein had on the organisms. The experiment regarding motor coordination was simple enough in theory. *C. elegans* are convenient in that when they are placed into any sort of liquid medium, they 'thrash' about--a defined and noticeable contraction about the midsection of the worm, bringing the head and tail together for a moment before relaxing and contracting again. To study how *atx-3* affected motor ability, all I simply had to do was count the worm's thrashes. However, there were a few complications to this I did not immediately foresee. In

short, at first the controls seemed difficult to guarantee: I needed a large sample number of each worm type. I needed to make sure I counted each worm's thrashes for the *exact* same amount of time, and I needed to ensure that the worms had the exact same conditions in between trials. Additionally, I struggled with counting the trashes in a 3-D environment, when my field of vision through the microscope was largely 2-D.

Originally, I played with the idea of cooling the worms and testing the M9 solution in which they would thrash, but that proved too difficult to control, and very time consuming. I also considered using agar plates similar to the ones with which our lines were maintained to do the experiments (in attempts to keep the worms in a 2-D environment that I could see more easily as compared to a large bubble of water), but (unsurprisingly), it was difficult to find the worms, and causing trouble with time. Eventually, I came up with a system that worked: with the consultation of Dr. Kikis, I found an interval app (intended for exercising) that I used to perfectly time both the adjustment period each organism was given before I began counting thrashes, and the following thrashing period. Because I no longer had to focus on keeping track of time itself, I could simply keep track of the organism's thrashes. I discovered the 3D/2D problem was largely eliminated by using a minimal amount of thrashing medium in which to place the worms. In short, I devised a completely testable experiment.

However, even with all these tools, it was still labor-intensive. I gave each worm a thirty-second adjustment period to get "acclimated" to the trashing media, and then counted the thrashes for a full 60 seconds. It does not sound that bad, until you take into consideration that thirty individuals were tested for each worm type, and there were four worm types. That already 180 minutes, not to factor in prep time: preparing the drops of thrashing medium on the slide, finding a suitable worm (e.g. right age, etc.) adjusting the scope, starting the timer, restarting the timer, and the experiment became an all-day event on staring into a microscope and pressing a counter. Perhaps something more frustrating (though to no one's fault), was that my labmates needed this microscope, too. I could not hog it all day. So, after a couple of mishaps and throwbacks, we successfully learned to communicate and coordinate very efficiently in order not to set anyone's experiments back. At the end, I had solid data that yielded significant results.

My other experiment, measuring the total amounts of *atx-3* protein in the worms, honestly sounds like the easier of the two experiments. In theory, it's super easy: in layman's terms, you collect some worms, boil them (sorry, worms) so that the proteins are accessible, and then run the mess through a gel, at which point all the proteins in the organisms separate according to size. In order to visualize the atx-3 protein (which would be denatured by the boiling and detergent that the gel is run in), the gel is transferred to a membrane, and antibodies (joined with YFP) are added that through one of several mechanisms binds to the denatured protein. In this way, the protein can be visualized.

Thing is, that wasn't working. It hadn't been working last semester, and it wasn't working during the summer. We tried different antibodies, we tried different amounts of worms---NOTHING worked. At some point, I realized that I had accidentally been switching the place of two steps in the (extremely well established) protocol, but even after that mistake had been corrected, I could not visualize the proteins in the gel. And to be honest it was extremely disheartening, seeing my experiment fail again and again and again (and it gets kind of boring, too). I was envious of my labmates, who were doing other types of protein measurements, and were doing it successfully. Eventually we combined the two protocols---the protocol I had been running with the protocol my labmates had been running for the experiments….and it worked. Of course, science loves certainly, so we have to run the experiment (again and again), but it was a HUGE step that we as a lab had needed for a long time.

I love the lab environment. I love how independent yet cooperative it is. Sure, I ran the experiments, but I learned so much from so many people: my lab coordinator, my labmates, and even people outside the field (such as Professor Rudd). The lab taught (and is teaching) me to learn to communicate and coordinate with all sorts of people to progress the research. I learned to maximize my time, planning out experiments that needed several days (if not weeks), and learning how to plan my experiments so I could do one experiment while waiting around for another. I am thoroughly convinced that research is what I want to go into---it's not work for me. It's like a fun, nerdy playground while I get to play with science, and hopefully better the world while I'm at it.